Adipose tissue

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CHAPTER 1

General introduction

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Increased prevalence of obesity and type 2 diabetes: A weighty problem

Until recently, it has been always a challenge for humans to find enough food to survive. For eras the ability to efficiently store energy during times of plenty and to economically use it during times of scarcity has been essential for survival. This phenomenon has put selective pressure on genes involved in food intake and energy storage, with gene variants that lead to efficient energy intake, usage and storage (also known as "thrifty genes") being favored over genes that are less economic (Neel 1962). Consequently, we nowadays find ourselves equipped with genes fit to an environment of regular energy shortage, while our current industrialized environment is the exact opposite. We tend to eat more calories than we burn and long periods of hunger are virtually absent in the Western World. As a result the human population is becoming heavier, and for the first time in history, overweight people outnumber the underweight: 1.4 billion people are overweight, against 800 million people that are underweight (Roth et al. 2004). Being overweight or obese accelerates the development of diseases that were formerly associated with ageing, such as type 2 diabetes, cardiovascular disease and certain types of cancers (reviewed in Roth et al. 2004; Brug & Crawford 2009).

Especially the prevalence of diabetes has increased substantially and doubled over the past 30 years. Currently, it is estimated that 7.6% of the world population suffers from type 2 diabetes (Roglic & World Health Organization 2016). About 60% of all type 2 diabetes cases are directly caused by overweight or obesity (World Health Organization 2000).The rapid emergence and occurrence of type 2 diabetes in children is particularly alarming (Roth et al. 2004). In The Netherlands, the costs for diabetes care were 1.7 billion euros in 2011, which is 1.9% of the total healthcare costs (RIVM 2013). In the USA, is has been estimated that out of every 10 dollars spend on healthcare, more than 1 dollar is spend on caring for people with diabetes (Petersen 2016). The increased prevalence of obesity and type 2 diabetes puts a huge socioeconomic burden on our society, which is only expected to rise over the next years (Baan et al. 2009).

It is evident that recent societal changes such as urbanization and industrialization are the main drivers of the recent obesity epidemic (World Health Organization 2000). However, not everyone in our urbanized and industrialized environment and/or with an unhealthy lifestyle habits becomes obese. This implies that in an obesogenic environment some individuals are more prone to develop obesity than others: Some possess a more "thrifty" gene set than others. The genes and gene variants that increase the risk of developing obesity are largely unidentified. This complicates our understanding of why some people become obese while others do

not. Also the mechanisms by which obesity leads to insulin resistance and type 2 diabetes are only partially resolved.

It is clear that excessive amounts of adipose tissue (the key characteristics of obesity) and adipose tissue dysfunction contribute to metabolic derangements (Goossens 2008). Vice versa, alleviation of adipose tissue dysfunction improves metabolic health, which makes the adipose tissue an attractive target for the treatment of metabolic diseases. In addition, the adipose secretes a wide variety of hormones, of which some have interesting metabolic properties (reviewed in Trayhurn & Wood, 2005). These adipokines provide new tools to further interrogate the complex mechanisms involved in metabolic regulation, and might provide new leads for the development of drugs. Therefore, studying adipose tissue biology helps us to understand the etiology of non-communicable disease and will aid in the identification of new drug targets that can be used to combat metabolic disorders.

Causes of overweight and obesity: Nature versus nurture

The most commonly used way to define obesity is by Body Mass Index (BMI). The BMI is calculated by dividing an individual's body weight by the square of his height (kg/m^2). Obesity is defined as a BMI equal to or greater than 30. The etiology of obesity is complex: The influence of the environment ("nurture") is evident, but there is also a strong genetic component ("nature") (World Health Organization 2000). Several studies showed that the heritability of BMI is about 70%, which underlines the notion that our genes are strong determinants of body weight (Stunkard et al. 1990; Wardle et al. 2008; Turula et al. 1990).

The heritability of body weight is high, but it is less clear which genes and variations therein determine our body weight. In humans only a handful of genes have been identified that, when mutated, inevitably lead to obesity. These include mutations in leptin and its receptor (LEP, LEP-R), pro-opiomelanocortin (POMC), melanocortin-4 receptor (MC4R), SH2 adapter protein 1 (SH2B1), pro-protein convertase 1 (PCSK1), and GNAS1 (GNAS). Their gene products are all involved in the central regulation of food intake and energy expenditure by the arcuate nucleus (ARC) in the hypothalamus (reviewed in O'Rahilly & Farooqi, 2000). The ARC contains two distinct populations of neurons: One expressing neuropeptide Y (NPY) and agouti-related protein (AgRP), the other expressing POMC and cocaine- and amphetamine-regulated transcript (CART). The NPY/AgRP neurons promote anabolic processes when activated, whereas the POMC/CART neurons promote catabolic processes (reviewed in Valassi, Scacchi, & Cavagnini, 2008). POMC is the precursor polypeptide for α-melanocyte-stimulating hormone (α-MSH), which is secreted into the synaptic cleft, where it binds to melanocortin-3 and melanocortin-4 receptors

on second-order neurons, which further activate catabolic pathways. Leptin, which is secreted by the adipose tissue, activates leptin receptors that are expressed by both the NPY/AgRP and the POMC/CART neurons, leading to inhibition of the former and stimulation of the latter, resulting in reduced food intake and increased energy expenditure (Cowley et al. 2001; Huo et al. 2009). (**Fig. 1**). Therefore, mutations in leptin, the leptin receptor, or SH2B1 (involved in the intracellular leptin signaling cascade), lead to defects in the leptin-induced activation of catabolic pathways.

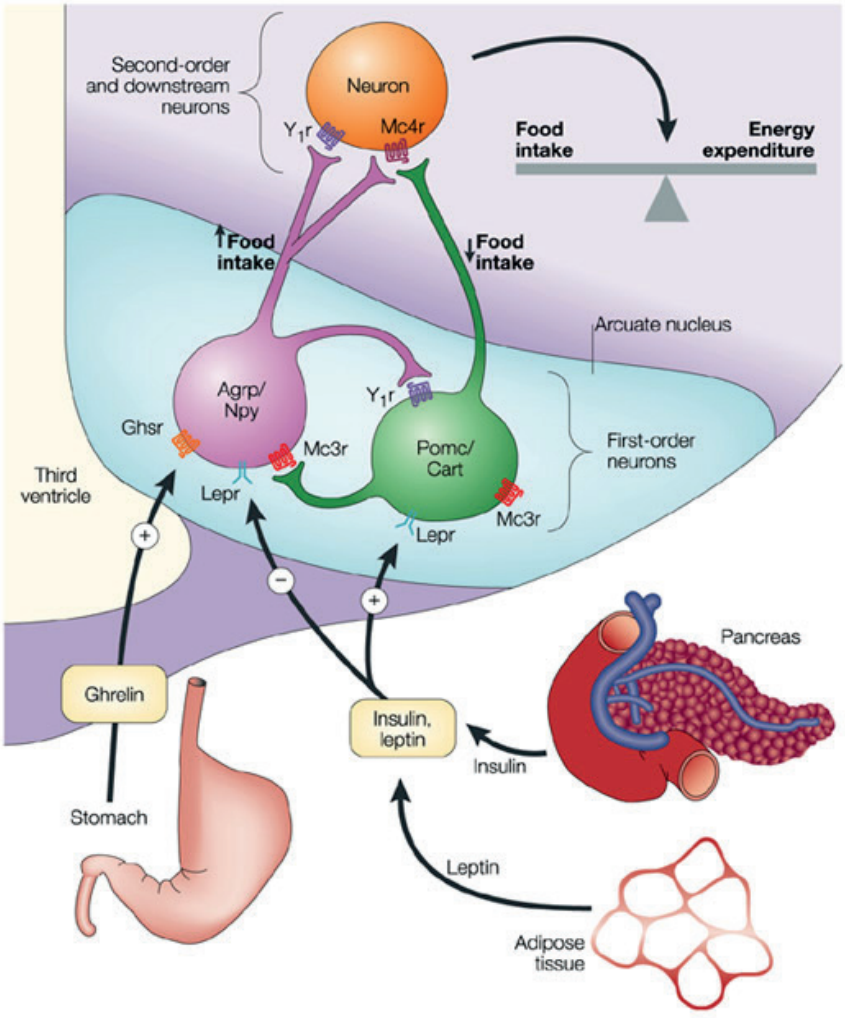

Figure 1. Regulation of food intake and energy expenditure by NPY/AgRP and POMC/CART expressing neurons in the hypothalamus. NPY/AgRP neurons stimulate food intake and reduce energy expenditure, POMC/CART neurons inhibit food intake and stimulate energy expenditure. Insulin and leptin inhibit AgRP/NPY neurons and stimulate POMC/CART neurons (Barsh & Schwartz 2002).

Likewise, mutations in POMC, the melanocortin-4 receptor, or proteins involved in posttranscriptional POMC processing such as PCSK1, results in hyperphagia and obesity. In mice, spontaneous mutations that lead to obesity include mutations in leptin (*ob/ob* mice) and the leptin receptor (*db/db* mice), AgRP (heterozygous lethal yellow mice), carboxypeptidase (*fat* mice), and tubby (*tubby* mice) (Coleman, 1978; Danforth, 1926; Coleman & Eicher, 1990). In addition, numerous other transgenic mice that develop obesity have been generated by knocking out or overexpressing genes involved in metabolism (reviewed in: Lutz & Woods, 2012).

Monogenic obesity (obesity caused by a mutation in a single gene) only explains a small percentage of the obesity development in our population. Most obesity cases are polygenic, in which variations in several genes determine the increased susceptibility for a high body weight. Mutations in these genes do not unequivocally lead to obesity, but rather increase the risk of becoming overweight, and are associated with a higher BMI (reviewed in: Hinney, Vogel, & Hebebrand, 2010).

Probably the best known gene is the fat mass and obesity associated gene *FTO*, which was identified through genome wide association studies (GWAS). People carrying both risk alleles weigh about 3 kg more and have a 1.67-fold increased odds of obesity compared to the ones not carrying the risk alleles (Frayling et al. 2007). The FTO protein is involved in mRNA processing and nutrient sensing, but how the mutated protein leads to obesity is still largely elusive (reviewed in: Hess & Brüning, 2014). Another gene that seems important for body weight control is *TUB*. Already in the nineties Stubdal and colleagues showed that a mutation in *TUB* was linked to metabolic regulation in mice. They showed that the *TUB* gene was responsible for the phenotype of the *tubby* mouse, a mouse strain that arose by spontaneous mutation in a mouse population the Jackson Laboratories (Coleman & Eicher 1990; Kleyn et al. 1996; Stubdal et al. 2000). *Tubby* mice show aberrations in food intake and energy expenditure, which leads to weight gain over time, late-onset obesity and a “tubby” appearance. In mice, *TUB* is expressed in several tissues and cell types, including the hypothalamus and adipocytes, but the functional mechanism by which mutations in *TUB* leads to obesity is incompletely understood (Prada et al. 2013; Ikeda et al. 2002; Sahly et al. 1998). In humans it has been shown that SNPs in *TUB* are associated with body weight and differences in macronutrient intake (Shiri-Sverdlov et al. 2006; Snieder et al. 2008; van Vliet-Ostaptchouk et al. 2008; van Tilburg et al. 2003). The exact expression pattern of *TUB* as well as its role in human metabolism remains unclear.

White adipose tissue: A key player in metabolic health and disease

The rise in obesity has boosted the interest in adipose tissue biology. The increase of our understanding of adipose tissue biology the past couple of decades has led to a paradigm shift: Whereas adipose tissue was originally regarded as a rather trivial passive organ, it is now viewed as an important active organ with high relevance to metabolic balance. It is a dynamic tissue that communicates with other organs through the controlled uptake, storage and release of energy, mainly in the form of free fatty acids (FFAs), and through the release of adipokines (reviewed in: Rosen & Spiegelman, 2014; Scherer, 2006). Adipose tissue is composed of several different cell types, including endothelial cells, pre-adipocytes, macrophages and other immune cells. Its main cell type, the mature adipocyte, is primarily responsible for the storage and release of energy and the secretion of adipokines (reviewed in: Trayhurn & Wood, 2005). Both processes are thought to be involved in the complex regulation of metabolism (Scherer, 2006).

WAT as an energy storage depot

Typical for the adipocyte is its ability to safely store large amounts of energy in the form of triglycerides during times of plenty, and to release energy by breaking down triglycerides into glycerol and FFAs during scarcity. Insulin is a major regulator of these two processes. It stimulates the uptake of FFAs into the adipocyte, where they are directly linked via a carboxyl ester to a glycerol backbone to form triglycerides. Insulin also stimulates the uptake of carbohydrates such as glucose, and their subsequent conversion into FFAs (which are then stored as triglycerides), a process called *de novo* lipogenesis (reviewed by Rosen & Spiegelman, 2014). The triglycerides are stored in lipid droplets, which also contain lipid droplet proteins that are involved in the regulation of triglyceride assembly and breakdown, such as perilipin (reviewed by Czech, Tencerova, Pedersen, & Aouadi, 2013). Insulin-stimulated glucose uptake is mediated via the phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, which induces the translocation of glucose transporter 4 (GLUT4) to the plasma membrane (reviewed by Rowland et al., 2011). Signaling through PI3K or the MEK-ERK pathway also leads to translocation of fatty acid transport protein 1 (FATP1) (Wu et al. 2006). The increased influx of glucose leads to activation of carbohydrate responsive element binding protein (ChREBP), which in turn stimulates the transcription of genes involved in lipogenesis, such as fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC) (Herman et al. 2012). Insulin inhibits lipolysis, the process in which triglycerides are hydrolyzed into glycerol and FFAs, which are subsequently released into the blood stream and used as energy substrates by other organs (reviewed by Duncan et al., 2007). There are three main lipases that control lipolysis: Adipose tissue triglyceride lipase (ATGL),

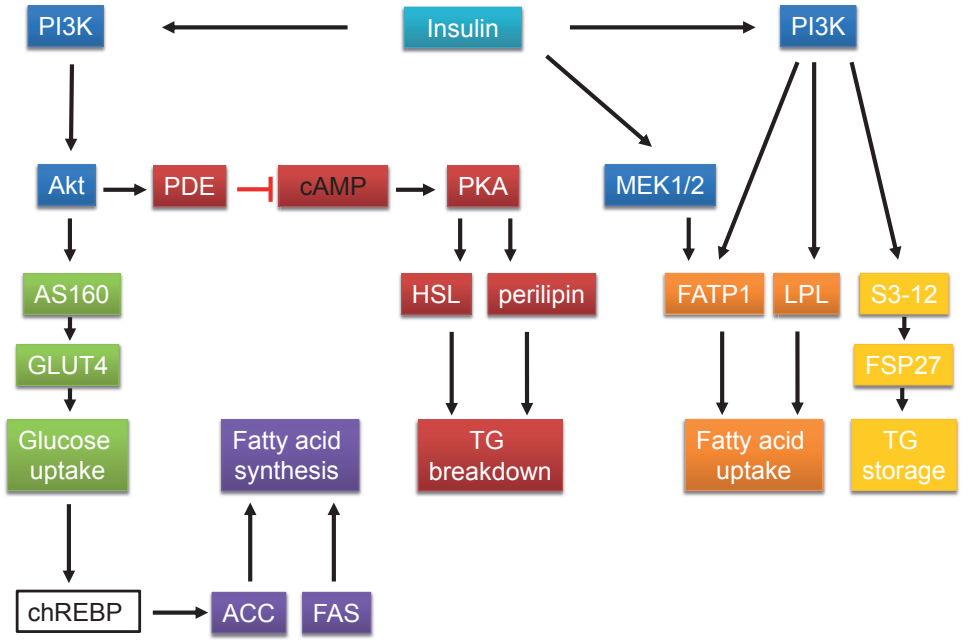

Figure 2. Insulin signaling and its effects on glucose and fatty acid uptake and storage (Adapted from Czech et al. 2013).

hormone-sensitive lipase (HSL) and monoacylglycerol lipase. Lipolytic stimuli, such as glucagon and norepinephrine, lead to increased intracellular cAMP levels and subsequent activation of protein kinase A (PKA) (reviewed in: Duncan et al., 2007; Rosen & Spiegelman, 2014). This facilitates the access of the lipases to the triglyceride droplet, resulting in increased lipolysis. Insulin activates phosphodiesterase 3B (PDE), a protein which reduces cAMP levels by converting cAMP into 5'AMP, leading to reduced PKA activity and reduced lipolytic activity by acting mainly on HSL and perilipin (reviewed in Czech et al., 2013; Morigny, Houssier, Mouisel, & Langin, 2016) (**Fig. 2**).

WAT as a secretory organ

Not many people know that adipose tissue forms the largest endocrine organ of the body. The secretion of adipose-derived factors, the adipokines, contributes significantly to the regulation of metabolism. The identification of leptin in 1994 was pivotal to the appreciation of the adipose as an endocrine tissue (Zhang et al. 1994). The subsequent discovery of leptin receptors in the hypothalamus, together with the observation that mice with a mutation in leptin or the leptin receptor suffer from hyperphagia and obesity, showed that the adipose tissue communicates to the brain via endocrine signals to regulate food intake and energy expenditure (Chen et

Table 1: Adipose tissue secretome (adjusted from www.themedicalbiochemistrypage.org)

Factor type:	
Growth- and angiogenic factors	fibroblast growth factors (FGFs), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), nerve growth factor (NGF), vascular endothelial cell growth factor (VEGF), transforming growth factor-β (TGFβ), angiopoietin-1, angiopoietin-2, tissue factor (TF, coagulation factor 3)
Cytokines	Interleukin-1beta (IL-1β), IL-4, IL-6, IL-8, IL-10, IL-18, tumor necrosis factor-alpha (TNFα), macrophage migration inhibitory factor (MIF)
Complement-like factors	adipsin, adiponectin, complement factor 3 (active form is C3adesArg, also known as acylation stimulating protein, ASP)
Chemokines	chemerin, monocyte chemotactic protein-1 (MCP-1), macrophage inhibitory protein-1 alpha (MIP-1α), chemokine (C-C motif) ligand 5 (CCL5)
Other	adipocyte fatty acid binding protein (FABP4, also known as aP2), apolipoprotein E (apoE), resistin, omentin, vaspin, apelin, retinol binding protein 4 (RBP4), visfatin, leptin

al. 1996; Tartaglia et al. 1995; Mercer et al. 1996). Another revolutionary discovery was the identification of adiponectin, an adipokine with insulin sensitizing, anti-atherogenic and anti-inflammatory properties (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995; Turer & Scherer, 2012). Adiponectin receptors are present on several tissues, including muscle, liver, adipose itself and several areas of the brain (Turer & Scherer 2012; Thundyil et al. 2012). Pharmacological administration of adiponectin either systemic or directly into the brain improves energy expenditure and insulin sensitivity (Qi et al. 2004; Park et al. 2011; Yamauchi et al. 2001). Since the discovery of leptin many other adipose-derived factors have been added to the adipokine list, which now comprises more than 50 different factors (reviewed in Trayhurn & Wood, 2005). These include growth factors, angiogenic factors, cytokines, complement-like factors, chemokines, and metabolic hormones (**Table 1**). Some of these peptides have beneficial effects, whereas other negatively influence metabolism.

Obesity, white adipose tissue and metabolic disease

A prolonged positive energy balance leads to increases in adipose tissue depots. This increase is primarily caused by increased triglyceride storage and lipogenesis (reviewed in Rosen & Spiegelman, 2014). The increase in adipocyte size is thought to be the main driver for adipose tissue dysfunction, and is thus tightly linked to the development of metabolic conditions such as type 2 diabetes. Large adipocytes are

less responsive to insulin (Salans et al. 1968), which leads to impaired repression of lipolysis and to high levels of FFAs in the circulation (reviewed in Rosen & Spiegelman, 2014). In addition, the increase in adipocyte size is thought to impair blood flow in the tissue, which leads to local hypoxia and changes in adipokine expression (reviewed in Trayhurn, 2014). Both abnormal FFA handling and altered adipokine secretion contribute to the development of metabolic diseases.

During obesity the FFAs that are present in high concentrations in the circulation are taken up by and stored ectopically in tissues such as the liver and muscle, where they contribute to insulin resistance (Shulman 2000; Donath & Shoelson 2011). There are several ways in which FFAs are thought to interfere with insulin signaling. It has been proposed that increased intracellular FFAs compete with glucose for substrate oxidation, and thereby impair glucose metabolism (Randle et al. 1963). The accumulation of "lipotoxic" lipid related molecules (such as diacylglycerol, fatty-acyl-CoA, and ceramides) can activate signaling pathways that lead to inhibitory phosphorylations on the insulin receptor substrates IRS-1 and IRS-2. As a consequence, downstream insulin signaling is impaired (reviewed in Shulman, 2000). This causes reduced glucose uptake into the skeletal muscle, and increased hepatic glucose output, two processes that contribute significantly to hyperglycemia (reviewed in Bergman & Ader, 2000). So although muscle and liver are the major tissues of insulin-responsive glucose turnover, their proper functioning is tightly linked to functional insulin signaling in adipose tissue. It has also been suggested that chronic exposure of pancreatic β-cells to FFAs results in β-cell dysfunction (reviewed by van Raalte et al., 2010).

Aberrant adipokine secretion is also though to contribute to metabolic aberratins during obesity. It has been shown that obesity is a state with altered adipokine secretion. Adiponectin, which is exclusively produced by the adipose tissue, is reduced (both in expression and in secretion) during the obese state (Arita et al. 1999). As adiponectin has insulin sensitizing properties and stimulates energy expenditure, it is thought that its downregulation during obesity exacerbates metabolic derangements (Yamauchi et al. 2001; Maeda et al. 2002). In contrast to adiponectin, the expression and secretion of leptin is increased during obesity (Maffei et al. 1995). It has been suggested that obesity leads to central leptin resistance (reduced sensitivity of the central nervous system for leptin) which causes an impaired satiety response, thereby aggravating the obese phenotype (Myers et al. 2010). Obesity-driven metabolic abnormalities can thus be caused by a reduced adipokine expression, or reduced adipokine sensitivity of the target tissue(s).

Obesity also drives the expression of pro-inflammatory cytokines such as TNFα, MCP-1 and IL-6, of which it is now generally accepted that they contribute significantly to the development of insulin resistance. These cytokines attract

macrophages which, when activated, also start to produce cytokines, leading to a vicious cycle of sustained low grade inflammation (reviewed in Lee, 2013). Adipose tissue inflammation has frequently been associated with insulin resistance. For example, it has been shown that TNFα is increased during obesity (Hotamisligil et al. 1993; Hotamisligil et al. 1995). TNFα knockout mice are protected against obesity-induced insulin resistance (Uysal et al. 1997). Another cytokine that has been associated with insulin resistance is MCP-1, which is increased in adipose tissue of obese subjects (Huber et al. 2008). Experiments in mice showed that infusion of MCP-1 induced insulin resistance, which could be reversed by blocking CCR2, the receptor for MCP-1 (Tateya et al. 2010).

White adipose tissue as a therapeutic target: TZDs and PPARγ

The fact that adipose tissue functions as a dynamic storage organ as well as an endocrine organ important for metabolism implies that the adipose tissue can be targeted to treat metabolic conditions. Indeed this is true, as the Thiazolidinedione (TZD) class of insulin sensitizers, which used to be widely prescribed to treat insulin resistance, mainly act via the adipose tissue. The glucose-lowering properties of this class of drugs was already discovered in 1988 (Fujiwara et al. 1988), but it took until 1995 to learn that TZDs act through activation of the nuclear receptor PPARγ (Lehmann et al. 1995). Around the same time it was found that FFAs form the natural ligands for PPARγ, and that PPARγ functions as the main regulator of adipocyte development and fat cell metabolism (Tontonoz et al. 1994; Graves et al. 1992). Subsequent studies showed that PPARγ is indispensable for adipose tissue development, maintenance and functioning, as it regulates adipocyte differentiation and fat storage (Tontonoz et al., 1994). Together these findings provided an important link between TZDs, PPARγ, adipose tissue function, and glucose metabolism. Activation of PPARγ with TZDs is very effective in improving insulin sensitivity and lowering blood glucose levels during type 2 diabetes, both in rodents and humans (Fujiwara et al. 1988; Nolan et al. 1994; Suter et al. 1992). The molecular mechanism by which TZDs mediate these effects are incompletely understood, but it is clear that adipose tissue plays a central role in their action, and that they influence both the storage capacity as well as the secretome of the adipose tissue (He et al. 2003)(reviewed in Tontonoz & Spiegelman, 2008).

TZDs stimulate both lipogenesis (storage of FFAs as triglycerides) in the adipose as well as adipogenesis, which is the process by which adipocyte precursor cells differentiate into mature adipocytes (Krotkiewski et al. 1983). TZDs increase the expression of genes involved in fatty acid uptake and storage (Tontonoz et al. 1994; Tontonoz et al. 1995; Martin et al. 1997). They induce differentiation of

pre-adipocytes into adipocytes, which appear as multiple small adipocytes in the adipose tissue (Okuno et al. 1998). TZDs stimulate uptake of FFAs into the adipose, lower circulating FFAs, and improve insulin sensitivity (Boden et al. 2005; Martin et al. 1997; Fujiwara et al. 1988). In general TZDs lead to increases in adipose tissue mass (reviewed in Soccio et al., 2014). Altogether this indicates that TZDs improve the "sink" function of the adipose, which leads to sequestration of FFAs away from the tissues where they impair insulin sensitivity, and towards the storage as more "safe" inert triglycerides in the white adipose tissue depots.

Another way by which pharmacological PPARγ activation leads to improved insulin sensitivity is via the modulation of adipokines. TZDs increase the expression and secretion of adiponectin in rodents and in humans (Maeda et al. 2001; Yu et al. 2002). The anti-diabetic effects of TZDs are blunted in adiponectin KO mice, which indicates that part of the insulin-sensitizing properties of TZDs are mediated by this adipokine (Nawrocki et al. 2006). In addition it has been shown that TZDs reduce inflammatory cytokines (Xu et al. 2003). However, the reduction in cytokines is mainly regulated via cells from the stromal-vascular fraction such as the macrophages (which also express PPARγ), and not by the adipocytes (Xu et al. 2003). In line with this notion, drugs that target the immune system are currently also under investigation for their properties to ameliorate glycemic control (reviewed in Kohlgruber & Lynch, 2015).

Although TZDs are very effective in establishing glycemic control they also cause serious side effects, which limits their clinical application (Lehrke & Lazar 2005; Tontonoz & Spiegelman 2008). Adverse effects include edema, osteoporosis and cardiovascular complications (Lehrke & Lazar 2005; Nissen & Wolski 2007). These side-effects are thought to be mediated by PPARγ activation in the adipose, kidney, and in cells involved in bone remodelling, such as the osteoblasts and osteoclasts (Guan et al. 2005; Lecka-Czernik 2010). TZD-associated fluid retention is particularly problematic, because it increases the risk of developing pulmonary edema and heart failure (Yang & Soodvilai 2008). Another side effect of the TZDs is weight gain due to increased adipogenesis and increased FFA storage in the adipocytes (reviewed in Soccio et al., 2014). However, as this leads to a reduction in ectopic fat accumulation, this weight gain is thought to contribute to the insulin sensitizing effects of the TZDs, which inseparably links the therapeutic effects of the TZDs to the side effect of modest increases in adipose tissue mass (reviewed in Lehrke & Lazar, 2005).

Adipose-derived factors as tools and therapeutic targets

The role of PPARγ in the mediation of insulin sensitivity is absolutely evident. However, to date relatively few PPARγ specific target genes that regulate metabolism have been identified. Therefore, the downstream effectors of PPARγ activation are largely unknown.

We recently discovered that PPARγ regulates the transcription of fibroblast growth factor 1 (FGF1) in adipocytes (Jonker et al. 2012). The expression of FGF1 is upregulated in adipocytes in response to high-fat diet feeding or TZD treatment. Remarkably, the FGF1 KO mouse has no obvious phenotype when kept under normal laboratory conditions and fed normal chow, which has led to the long-held assumption that FGF1 is an irrelevant FGF (reviewed in Beenken & Mohammadi, 2009). However, when fed a high-fat diet the FGF1 KO mouse displays a phenotype characterized by impaired adipose tissue expansion, ectopic lipid accumulation, and severe insulin resistance (Jonker et al. 2012). This shows that FGF1 mediates part of the effects of PPARγ activation, and is indispensable for WAT expansion and energy storage in response to high fat-diet feeding, and thus to metabolic homeostasis during times of energy abundance. How endogenous FGF1 regulates metabolic processes is still elusive, but as FGF1 is thought to mainly act as an autocrine or paracrine factor (reviewed in Nies et al. 2015), it is assumed that it works directly on the adipocyte itself or on cells in its vicinity, such as endothelial cells or macrophages. Indeed it has been shown that FGF1 promotes pre-adipocyte proliferation and differentiation (Hutley et al. 2004; Newell et al. 2006), and that it promotes angiogenesis (Murakami & Simons 2008). Whether and how FGF1 regulates metabolic processes in adipocytes is currently unknown.

There are four different FGF receptors (FGFR1-FGFR4). FGF1 can bind to and activate all four of these receptors (Eswarakumar et al. 2005). Activation of FGF receptors leads to the activation of three several downstream signaling pathways: Phospholipase C γ (PLCγ) signaling, MEK-ERK signaling, and PI3K-Akt signaling (reviewed in Nies et al. 2015). The contribution of each signaling pathway to the metabolic effects of FGFs remains yet to be revealed.

We further demonstrated that pharmacological treatment of obese, hyperglycemic rodents with recombinant FGF1 recapitulates part of the effects of TZD administration: Similar to TZD administration, FGF1 treatment induces potent glucose lowering and insulin-sensitizing effects at the systemic level in obese mice (Suh et al. 2014). These effects are dependent on the presence of FGFR1 on the adipocytes (Suh et al. 2014). FGF1 treatment also effectively reduces hepatic steatosis (Liu et al. 2016). The results of FGF1 treatment show remarkable overlap with those obtained upon TZD treatment. However, FGF1 treatment does not lead to weight gain, edema or bone loss, indicating that FGF1 improves metabolism

similar to pharmacological PPARγ activation, but without provoking many of the negative side effects that are associated with TZD administration (Suh et al. 2014). The mechanism by which FGF1 improves blood glucose levels is currently unknown, but its metabolic properties put FGF1 forward as an interesting candidate for the development of FGF1 based drugs.

The pharmaceutical potential of FGFs can be illustrated by FGF21, a hormone-like FGF whose metabolic properties were discovered in an *in vitro* screen about ten years ago (Kharitonenkov et al. 2005). FGF21 was initially identified as a liver-derived hormone, whose expression is under transcriptional control of PPARα (Nishimura et al. 2000; Badman et al. 2007). Pharmacological administration of FGF21 greatly improves the metabolic profile of obese rodents, an effect which (like FGF1) is largely dependent on FGFR1 signaling in the adipose (Kharitonenkov et al. 2005; Adams et al. 2013). These effects include lowering of plasma glucose, triglycerides and insulin sensitization (Adams et al. 2013). FGF21 also induces weight loss and increases energy expenditure, but these effects are thought to be primarily mediated via the brain (Owen et al. 2014). Some FGF21-based drugs are already being tested for their efficacy in humans (Gaich et al., 2013).

Aim and outline of the thesis

In the past decades great effort and progress has been made in understanding how and why we become obese. It has become clear that there is a strong genetic component to the development of obesity, but the genes and processes that lead to increased susceptibility for weight gain and excessive fat accumulation are poorly identified. Adipose tissue has an important role in the regulation of metabolism, and is a key player in the etiology of obesity and type 2 diabetes. As a result, the adipose tissue has become an attractive therapeutic target for the treatment of these two conditions. The discovery that the adipose secretes a variety of compounds that are involved in metabolic regulation, has generated hope that the adipose secretome can provide new starting points for the understanding of metabolism, and the development of treatments against metabolic diseases. The adipose tissue is therefore not only a therapeutic target, it also functions as a “toolbox” that holds novel tools that we can use to interrogate metabolic processes and to design new drugs.

The scope of this thesis comprises two factors that are expressed in the adipose tissue: FGF1 and TUB. FGF1 is an example of an adipose-derived peptide, whose physiological role involves coordination of the response of the adipose tissue to feeding and fasting cycles (Jonker et al. 2012). In addition it possesses potent

pharmacological properties when administered to mouse models of obesity and type 2 diabetes, which are largely dependent of FGFR1 signaling in the adipose (Suh et al. 2014). FGF1 is thus a tool to better understand the adaptive response to feeding and fasting, and forms a novel therapeutic lead for the treatment of metabolic conditions.

TUB is a gene that has been associated with obesity development both in mice and in humans. In mice, *TUB* is expressed in several areas of the hypothalamus and in adipocytes (Prada et al. 2013; Ikeda et al. 2002; Sahly et al. 1998). The distribution and expression level of *TUB* in humans in metabolically relevant tissues such as the adipose are largely unexplored.

The **main aim** of this thesis is to **provide insight into physiological and pharmacological properties of FGF1**, including the functional mechanism(s) by which FGF1 exerts its effects (chapter 2, 3, 4 and 5). The **second aim** is to **gain a better understanding of *TUB* in humans** (chapter 6).

In **chapter 2** we review the current knowledge on FGFs in metabolic health and disease. We describe the current insights in FGF-related research and recent developments in FGF-based drug design. In addition we provide new possible leads for the development of FGF-based treatment options against metabolic conditions.

FGF1 has a high affinity for heparan sulphate proteoglycans, which largely prevent the escape of the endogenously produced factor from the adipose tissue into the circulation (Mejhert et al. 2010; Beenken & Mohammadi 2009). Therefore it is assumed that FGF1 works as an autocrine or paracrine factor. It is unknown how FGF1 affects metabolic processes in mature adipocytes. Also the intracellular signaling pathway that mediates its effects is currently unknown. Because of the profound role of FGF1 in the regulation of glucose metabolism and its evident dependence on adipose tissue, we examined the effects of FGF1 on glucose uptake in adipocytes, which is described in **chapter 3**. We used the 3T3-L1 adipocyte cell line in combination with pharmacological inhibitors to interrogate signaling pathways involved in FGF1-mediated glucose uptake, and investigated its effects on insulin-stimulated glucose transport.

So far most of the research regarding FGF1 has been performed in mouse models of type 2 diabetes, in which it has been shown that FGF1 improves glycemic control (Suh et al. 2014). This effect is dependent on the presence of insulin, as FGF1 did not have this effect in mice rendered insulin-depleted, either by STZ-induced β-cell loss or by temporary somatostatin infusion. In addition, FGF1 is unable to lower blood glucose levels of normoglycemic, chow-fed wild-type mice (Suh et al. 2014). This implies that the blood glucose lowering properties of FGF1 depend on a metabolic

condition that is present in *ob/ob* mice or DIO mice, such as hyperglycemia or insulin resistance.

Whether FGF1 also has insulin sensitizing effects in T1D is elusive. Moreover it is unknown whether the insulin sensitizing properties of FGF1 rely on the presence of insulin resistance, hyperglycemia, or both. Therefore, we determined the effects FGF1 on the blood glucose lowering properties of insulin in three different models of T1D: the alloxan-induced diabetic mouse, the streptozotocin (STZ)-induced diabetic mouse, and the Non-Obese Diabetic (NOD)-mouse. The results of this study are described in **chapter 4**.

It is thought that pharmacological administered FGF21 lowers blood glucose levels of type 2 diabetic rodents by repressing the hepatic glucose production and by stimulating glucose uptake into the adipose tissue (Berglund et al. 2009; Ding et al. 2012). Whether FGF1 functions via a similar mechanism is unknown. Also the intracellular signaling pathways that mediate the metabolic effects of FGF1 are currently elusive. In **chapter 5** we investigated the acute effects of FGF1 *in vivo* in obese, hyperglycemic mice. We used isotope and radioactive tracer techniques to assess whole body- and tissue specific glucose metabolism. We investigated PI3K-Akt and MEK-ERK signaling in several metabolically relevant tissues, and used a MEK inhibitor to determine the contribution of the MEK-ERK signaling pathway to the metabolic properties of FGF1.

Despite its unmistakable relevance in the development of obesity in mice, the role of *TUB* in human obesity is largely unexplored. In mice, *TUB* is expressed in the testis, skeletal muscle, heart and adipocytes, and in several hypothalamic areas involved in food intake and metabolism (Sahly 1998; Stretton et al. 2009; Prada et al. 2013; Ikeda et al. 2002). The expression and distribution of *TUB* in the human hypothalamus has not been explored yet. It is also unknown how *TUB* is expressed in the human adipose tissue. As mutations in *TUB* are associated with obesity, we hypothesized that differences in *TUB* gene expression either in the hypothalamus and/or the adipose tissue might correlate with indices of obesity. Therefore in **chapter 6** we determined the distribution and expression of *TUB* in the hypothalamus and adipose tissues of humans, and analyzed whether or not its expression was associated with indices of metabolic health and body weight.

In **chapter 7** we discuss the major findings described in this thesis, and give recommendations for future research.

References

Adams, A.C. et al., 2013. The breadth of FGF21's metabolic actions are governed by FGFR1 in adipose tissue. Molecular metabolism, 2(1), pp.31–7.

Arita, Y. et al., 1999. Paradoxical Decrease of an Adipose-Specific Protein, Adiponectin, in Obesity. Biochemical and Biophysical Research Communications, 257(1), pp.79–83.

Baan, C.A. et al., 2009. Diabetes to 2025: Preventie en zorg in samenhang,

Badman, M.K. et al., 2007. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell metabolism, 5(6), pp.426–37.

Barsh, G.S. & Schwartz, M.W., 2002. Genetic approaches to studying energy balance: perception and integration. Nature Reviews Genetics, Published online: 01 August 2002; | doi:10.1038/nrg862, 3(8), p.589.

Beenken, A. & Mohammadi, M., 2009. The FGF family: biology, pathophysiology and therapy. Nature reviews. Drug discovery, 8(3), pp.235–253.

Berglund, E.D. et al., 2009. Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. Endocrinology, 150(9), pp.4084–93.

Bergman, R.N. & Ader, M., 2000. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends in endocrinology and metabolism: TEM, 11(9), pp.351–6.

Boden, G. et al., 2005. Thiazolidinediones upregulate fatty acid uptake and oxidation in adipose tissue of diabetic patients. Diabetes, 54(3), pp.880–5.

Brug, J. & Crawford, D., 2009. The obesity pandemic. Is it bad or worse? The European Journal of Public Health, 19(6).

Chen, H. et al., 1996. Evidence That the Diabetes Gene Encodes the Leptin Receptor: Identification of a Mutation in the Leptin Receptor Gene in db/db Mice. Cell, 84(3), pp.491–495.

Coleman, D.L., 1978. Obese and diabetes: Two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia, 14(3), pp.141–148.

Coleman, D.L. & Eicher, E.M., 1990. Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. The Journal of heredity, 81(6), pp.424–7.

Cowley, M.A. et al., 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature, 411(6836), pp.480–484.

Czech, M.P. et al., 2013. Insulin signalling mechanisms for triacylglycerol storage. Diabetologia, 56(5), pp.949–64.

Danforth, C.H., 1926. Hereditary adiposity in mice. The Journal of heredity, 29, pp.153–162.

Ding, X. et al., 2012. βKlotho is required for fibroblast growth factor 21 effects on growth and metabolism. Cell metabolism, 16(3), pp.387–93.

Donath, M.Y. & Shoelson, S.E., 2011. Type 2 diabetes as an inflammatory disease. Nature reviews. Immunology, 11(2), pp.98–107.

Duncan, R.E. et al., 2007. Regulation of lipolysis in adipocytes. Annual review of nutrition, 27, pp.79–101.

Eswarakumar, V., et al., 2005. Cellular signaling by fibroblast growth factor receptors. Cytokine & Growth Factor Reviews, 16(2), pp.139–149.

Frayling, T.M. et al., 2007. A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. Science, 316(5826), pp.889–894.

Fujiwara, T. et al., 1988. Characterization of New Oral Antidiabetic Agent CS-045: Studies in KK and ob/ob Mice and Zucker Fatty Rats. Diabetes, 37(11).

Gaich G, et al. 2013. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2

diabetes. Cell Metabolism;18(3):333-40. doi:10.1016/j.cmet.2013.08.005

Goossens, G.H., 2008. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiology & Behavior, 94(2), pp.206–218.

Graves, R.A., et al., 1992. Analysis of a tissue-specific enhancer: ARF6 regulates adipogenic gene expression. Molecular and cellular biology, 12(3), pp.1202–8.

Guan, Y. et al., 2005. Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption. Nature Medicine, 11(8), pp.861–866.

He, W. et al., 2003. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proceedings of the National Academy of Sciences of the United States of America, 100(26), pp.15712–7.

Herman, M.A. et al., 2012. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature, 484(7394), pp.333–338.

Hess, M.E. & Brüning, J.C., 2014. The fat mass and obesity-associated (FTO) gene: Obesity and beyond? Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1842(10), pp.2039–2047.

Hinney, A., et al., 2010. From monogenic to polygenic obesity: recent advances. European child & adolescent psychiatry, 19(3), pp.297–310.

Hotamisligil, G.S. et al., 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. The Journal of clinical investigation, 95(5), pp.2409–15.

Hotamisligil, G.S., et al., 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science (New York, N.Y.), 259(5091), pp.87–91.

Huber, J. et al., 2008. CC Chemokine and CC Chemokine Receptor Profiles in Visceral and Subcutaneous Adipose Tissue Are Altered in Human Obesity. The Journal of Clinical Endocrinology & Metabolism, 93(8), pp.3215–3221.

Huo, L. et al., 2009. Leptin-Dependent Control of Glucose Balance and Locomotor Activity by POMC Neurons. Cell Metabolism, 9(6), pp.537–547.

Hutley, L. et al., 2004. Fibroblast Growth Factor 1: A Key Regulator of Human Adipogenesis. Diabetes, 53(12), pp.3097–3106.

Ikeda, A. et al., 2002. The tubby-like proteins, a family with roles in neuronal development and function. J. Cell Sci., 115(1), pp.9–14.

Jonker, J.W. et al., 2012. A PPARγ–FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. Nature, 485(7398), pp.391–394.

Kharitonenkov, A. et al., 2005. FGF-21 as a novel metabolic regulator. The Journal of clinical investigation, 115(6), pp.1627–35.

Kleyn, P.W. et al., 1996. Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. Cell, 85(2), pp.281–90.

Kohlgruber, A. & Lynch, L., 2015. Adipose Tissue Inflammation in the Pathogenesis of Type 2 Diabetes. Current Diabetes Reports, 15(11), p.92.

Krotkiewski, M. et al., 1983. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. The Journal of clinical investigation, 72(3), pp.1150–62.

Lecka-Czernik, B., 2010. Bone Loss in Diabetes: Use of Antidiabetic Thiazolidinediones and Secondary Osteoporosis. Current Osteoporosis Reports, 8(4), pp.178–184.

Lee, J., 2013. Adipose tissue macrophages in the development of obesity-induced inflammation, insulin resistance and type 2 Diabetes. Archives of Pharmacal Research, 36(2), pp.208–222.

Lehmann, J.M. et al., 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). The Journal of biological chemistry, 270(22), pp.12953–6.

Lehrke, M. & Lazar, M.A., 2005. The many faces of PPARgamma. Cell, 123(6), pp.993–9.

Liu, W. et al., 2016. Effective treatment of steatosis and steatohepatitis by fibroblast growth factor 1 in mouse models of nonalcoholic fatty liver disease. Proceedings of the National Academy of Sciences of the United States of America, 113(8), pp.2288–93.

Lutz, T.A. & Woods, S.C., 2012. Overview of animal models of obesity. Current protocols in pharmacology, Chapter 5, p.Unit5.61.

Maeda, N. et al., 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature Medicine, 8(7), pp.731–737.

Maeda, N. et al., 2001. PPARγ Ligands Increase Expression and Plasma Concentrations of Adiponectin, an Adipose-Derived Protein. Diabetes, 50(9).

Maffei, M. et al., 1995. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature Medicine, 1(11), pp.1155–1161.

Martin, G. et al., 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. The Journal of biological chemistry, 272(45), pp.28210–7.

Mejhert, N. et al., 2010. Mapping of the fibroblast growth factors in human white adipose tissue. The Journal of clinical endocrinology and metabolism, 95(5), pp.2451–7.

Mercer, J.G. et al., 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Letters, 387(2), pp.113–116.

Morigny, P. et al., 2016. Adipocyte lipolysis and insulin resistance. Biochimie, 125, pp.259–266.

Murakami, M. & Simons, M., 2008. Fibroblast growth factor regulation of neovascularization. Current opinion in hematology, 15(3), pp.215–20.

Myers, M.G. et al., 2010. Obesity and leptin resistance: distinguishing cause from effect. Trends in endocrinology and metabolism: TEM, 21(11), pp.643–51.

Nawrocki, A.R. et al., 2006. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. The Journal of biological chemistry, 281(5), pp.2654–60.

Neel, J. V, 1962. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? American journal of human genetics, 14(4), pp.353–62.

Newell, F.S. et al., 2006. Characterization of the transcriptional and functional effects of fibroblast growth factor-1 on human preadipocyte differentiation. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 20(14), pp.2615–2617.

Nies, V.J.M. et al., 2015. Fibroblast Growth Factor Signaling in Metabolic Regulation. Frontiers in endocrinology, 6, p.193.

Nishimura, T. et al., 2000. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression, 1492(1), pp.203–206.

Nissen, S.E. & Wolski, K., 2007. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. New England Journal of Medicine, 356(24), pp.2457–2471.

Nolan, J.J. et al., 1994. Improvement in Glucose Tolerance and Insulin Resistance in Obese Subjects Treated with Troglitazone. New England Journal of Medicine, 331(18), pp.1188–1193.

O'Rahilly, S. & Farooqi, I.S., 2000. The Genetics of Obesity in Humans, MDText.com, Inc.

Okuno, A. et al., 1998. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. The Journal of clinical investigation, 101(6), pp.1354–61.

Owen, B.M. et al., 2014. FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. Cell metabolism, 20(4), pp.670–7.

Park, S. et al., 2011. Long-Term Central Infusion of Adiponectin Improves Energy and Glucose Homeostasis by Decreasing Fat Storage and Suppressing Hepatic Gluconeogenesis without Changing

Food Intake. Journal of Neuroendocrinology, 23(8), pp.687–698.

Petersen, M., 2016. Economic costs of diabetes in the U.S. in 2012. Diabetes Care, 39(7), pp.1033–1046.

Prada, P.O. et al., 2013. Tub Has a Key Role in Insulin and Leptin Signaling and Action In Vivo in Hypothalamic Nuclei. Diabetes, 62(1), pp.137–148.

Qi, Y. et al., 2004. Adiponectin acts in the brain to decrease body weight. Nature Medicine, 10(5), pp.524–529.

van Raalte, D.H. et al., 2010. Pancreatic steatosis in humans: cause or marker of lipotoxicity? Current opinion in clinical nutrition and metabolic care, 13(4), pp.478–85.

Randle, P.J. et al., 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet (London, England), 1(7285), pp.785–9.

RIVM, 2013. Kosten van Ziekten in Nederland. , p.kvz_v2l1b1p4r0c0i0t1j0o3y6a-1g0d25s54f0z0w2. Available at: https://kostenvanziektentool.volksgezondheidenzorg.info/tool/nederlands/?ref=kvz_v2l1b1p4r4c2i0t1j0o1y6a-1g0d25s54z0f0w2 [Accessed November 28, 2016].

Roglic, G. & World Health Organization, 2016. Global report on diabetes

Rosen, E.D. & Spiegelman, B.M., 2014. What we talk about when we talk about fat. Cell, 156(1–2), pp.20–44.

Roth, J. et al., 2004. The Obesity Pandemic: Where Have We Been and Where Are We Going? Obesity Research, 12(S11), p.88S–101S.

Rowland, A.F. et al., 2011. Mapping insulin/GLUT4 circuitry. Traffic (Copenhagen, Denmark), 12(6), pp.672–81.

Sahly, I., 1998. Prominent neuronal-specific tub gene expression in cellular targets of tubby mice mutation. Human Molecular Genetics, 7(9), pp.1437–1447.

Salans, L.B. et al., 1968. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. The Journal of clinical investigation, 47(1), pp.153–65.

Scherer, P.E. et al., 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. Journal of Biological Chemistry, 270(45), pp.26746–26749.

Scherer, P.E., 2006. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes, 55(6), pp.1537–1545.

Shiri-Sverdlov, R. et al., 2006. Identification of TUB as a novel candidate gene influencing body weight in humans. Diabetes, 55(2), pp.385–9.

Shulman, G.I., 2000. On diabetes : insulin resistance Cellular mechanisms of insulin resistance. , 106(2), pp.171–176.

Snieder, H. et al., 2008. TUB is a candidate gene for late-onset obesity in women. Diabetologia, 51(1), pp.54–61.

Soccio, R.E. et al., 2014. Thiazolidinediones and the Promise of Insulin Sensitization in Type 2 Diabetes. Cell Metabolism, 20(4), pp.573–591.

Stretton, C. et al., 2009. Expression and modulation of TUB by insulin and thyroid hormone in primary rat and murine 3T3-L1 adipocytes. Biochemical and biophysical research communications, 390(4), pp.1328–33.

Stubdal, H. et al., 2000. Targeted deletion of the tub mouse obesity gene reveals that tubby is a loss-of-function mutation. Molecular and cellular biology, 20(3), pp.878–82.

Stunkard, A.J. et al., 1990. The Body-Mass Index of Twins Who Have Been Reared Apart. New England Journal of Medicine, 322(21), pp.1483–1487.

Suh, J.M. et al., 2014. Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer. Nature.

Suter, S.L. et al., 1992. Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects.

Diabetes care, 15(2), pp.193–203.

Tartaglia, L.A. et al., 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell, 83(7), pp.1263–1271.

Tateya, S. et al., 2010. An increase in the circulating concentration of monocyte chemoattractant protein-1 elicits systemic insulin resistance irrespective of adipose tissue inflammation in mice. Endocrinology, 151(3), pp.971–9.

Thundyil, J. et al., 2012. Adiponectin receptor signalling in the brain. British Journal of Pharmacology, 165(2), pp.313–327.

van Tilburg, J.H. et al., 2003. Genome-wide screen in obese pedigrees with type 2 diabetes mellitus from a defined Dutch population. European journal of clinical investigation, 33(12), pp.1070–4.

Tontonoz, P. et al., 1995. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol. Cell. Biol., 15(1), pp.351–357.

Tontonoz, P., Hu, E. & Spiegelman, B.M., 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell, 79(7), pp.1147–1156.

Tontonoz, P. & Spiegelman, B.M., 2008. Fat and Beyond: The Diverse Biology of PPARγ. Annual Review of Biochemistry, 77(1), pp.289–312.

Trayhurn, P., 2014. Hypoxia and adipocyte physiology: implications for adipose tissue dysfunction in obesity. Annual review of nutrition, 34, pp.207–36.

Trayhurn, P. & Wood, I.S., 2005. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochemical Society Transactions, 33(5).

Turer, A.T. & Scherer, P.E., 2012. Adiponectin: mechanistic insights and clinical implications. Diabetologia, 55(9), pp.2319–2326.

Turula, M. et al., 1990. Body weight in the Finnish Twin Cohort. Diabetes research and clinical practice, 10 Suppl 1, pp.S33-6.

Uysal, K.T. et al., 1997. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature, 389(6651), pp.610–4.

Valassi, E. et al., 2008. Neuroendocrine control of food intake. Nutrition, Metabolism and Cardiovascular Diseases, 18(2), pp.158–168.

van Vliet-Ostaptchouk, J. V et al., 2008. Polymorphisms of the TUB gene are associated with body composition and eating behavior in middle-aged women. PloS one, 3(1), p.e1405.

Wardle, J. et al., 2008. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. The American journal of clinical nutrition, 87(2), pp.398–404.

World Health Organization, 2000. Obesity: Preventing and managing the global epidemic - report of a WHO consultation. , p.268.

Wu, Q. et al., 2006. FATP1 Is an Insulin-Sensitive Fatty Acid Transporter Involved in Diet-Induced Obesity. Molecular and Cellular Biology, 26(9), pp.3455–3467.

Xu, H. et al., 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of clinical investigation, 112(12), pp.1821–30.

Yamauchi, T. et al., 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature Medicine, 7(8), pp.941–946.

Yang, T. & Soodvilai, S., 2008. Renal and vascular mechanisms of thiazolodinedione-induced fluid retention. PPAR research .

Yu, J.G. et al., 2002. The Effect of Thiazolidinediones on Plasma Adiponectin Levels in Normal, Obese, and Type 2 Diabetic Subjects. Diabetes, 51(10).

Zhang, Y. et al., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature, 372(6505), pp.425–432.